BIOLOGIC STUDIES OF THE DIPHTHERIA BACILLUS

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1. THE SPECIFICITY OF THE MORPHOLOGIC TYPES

There has been considerable uncertainty evidenced concerning the variability of the morphologic characteristics of B. diphtheriae with reference to Wesbrook's types. The general opinion appears to be that these types are to a great extent, at least, immutable. If a particular form is found it is assumed that the strain remains constantly of that type of morphology. It has, furthermore, been assumed that the solid staining forms, D_2 and E_2, are relatively avirulent strains, and are usually reported avirulent or even negative in diagnostic laboratory reports.

The literature on the subject is not by any means in accord with these views. Almqvist and Koraen [1] report observations of one strain of diphtheria bacillus over several years, and they found no marked change in its morphology at any examination. However, Denny [2] found that strains of types C_2 and D_2 tend, on prolonged cultivation, to become clubbed and to stain irregularly; in other words, to approach the barred and granular forms in their staining properties. He concludes that the solid types are young forms. Wherry[3] arrives at the same conclusion from a study of the retarded growth of the organism under conditions of lowered oxygen tension. When growth takes place under such conditions it is retarded, and only the solid staining forms are found, principally D_2 and E_2. However, if such a culture is allowed to grow at normal oxygen tension barred and granular types soon develop.

In connection with other problems, the question of the specificity of the morphology arose and studies were consequently made to determine whether variations in morphology and staining properties were common and if so, under what conditions these changes take place. Accordingly, a detailed study has been made of a series of strains with reference to their morphologic characteristics, to determine the specific or nonspecific nature of their morphology and the

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[1] Ztschr. f. Hyg. u. Infektionskr., 1918, 85, p. 347.
[2] Jour. Med. Research, 1913, 9, p. 117.
[3] Jour. Infect. Dis., 1917, 9, p. 27.

relation of morphologic characteristics to certain biologic properties, such as virulence, toxin production and agglutinability. These strains were obtained through the courtesy of R. L. Laybourn, bacteriologist of the Iowa State Board of Health.

The bipolar or granular types, C and possibly D of Wesbrook's classification, have been regarded as the typical form of the diphtheria bacillus and are the forms, together with type A, most frequently encountered. Type A, composed of organisms with clubbed or swollen ends, are not so often found, and have been regarded as involution forms. The barred forms are more rare, but are usually considered typical virulent forms, with the exception of those which fall into types E_1 and F, which are usually found to be of questionable virulence.

In a study of 154 strains of the diphtheria bacillus the relative numbers of the different morphologic types have been noted, their tendency to remain constant has been studied and the relation of the culture to its morphology has been considered.

It is impossible to make a definite statement of the relative proportions of the several types as they occur under natural conditions as it has been found that in a considerable number of cases the age of the culture determines the morphology of the organisms. This is illustrated in the following series of cultures studied.

The cultures obtained were streaked on blood serum plates and individual colonies isolated and subcultured at the end of 4 to 8 hours. These were examined for morphology and then transferred to slants of the same medium for further study.

TABLE 1

RELATIONSHIP OF MORPHOLOGY TO AGE OF CULTURES

Culture	4 Hours	8 Hours	24 Hours	Subculture, 4 Hours
21	D_2	D_2	C	D_2
124	D_2	D_2	C	D_2
168	D_2	C and D_2	C	D_2
215	E_2	E_2 and C	C	E_2 and D_2
216	D_2	C	C	D_2
218	D_2	D_2	C	D_2
219	E_2	E_2 and C	C	E_2
220	D_2	D_2	C	D_2
224	E_2	E_2	C	E_2

From the table it is evident that 9 strains of the series, or about 6%, were of the solid types at the end of short periods of incubation, but at the end of 24 hours they had assumed the typical granular appearance. The morphology and staining characteristics of the so-called atypical bacilli had changed to that of the typical virulent organism. In most cases a period of from 12 to 24 hours was required to produce the change from the solid type to the granular and, as appears in the table, all of these 9 strains on subculture reverted to the solid form.

That the morphologic characteristics of the solid forms are not always reversible is shown in Table 2. The strains in this series, having once become granular, remained so in subsequent subcultures at all examinations.

TABLE 2

GRANULAR AND BIPOLAR FORMS INITIALLY OF SOLID TYPES

Culture	4 Hours	12 Hours	24 Hours	Subculture, 4 Hours
181	D_2	D_2	C	C
209	D_2	D_2	D	D
211	E_2	E_2	C	C
214	D_2	D_2	C	C
217	E_2	E_2	C	C
222	D_2	D_2	D	D

Further subculture showed that these strains remain of the bipolar or granular types indefinitely.

This apparent change in types has usually been from solid to granular. However, 3 strains were encountered in this series which were type C forms initially but which on further subculture were found to be solid staining forms, D_2 or E_2. The most striking tendency noted in this study has been a change from the types which are assumed to be of low virulence to the typically virulent types of the organism. Of 19 originally solid forms, it will be seen from tables 1 and 2 that 15 were found to become granular or bipolar on prolonged cultivation. Four strains remained solid at all examinations.

That changes due to the growth of the organisms are a factor in determining morphology and staining properties seems possible. A study of these conditions is being made at the present time in this laboratory and will be made the subject of a future report.

From an epidemiologic standpoint it is improbable that the morphologic types of the diphtheria bacillus are biologically specific. Unlike the types of the pneumococcus and meningococcus which rarely occur but singly in the nasopharynx, the cultures of the nasopharynx in active cases and carriers of diphtheria regularly show a mixture of morphologic forms. The predominating type in the active case has no bearing on the type found in a contact.

The observations here recorded corroborate the findings reported by other investigators that the solid staining types are young forms that later develop the granules and polar bodies characteristic of the other types. If this is the case, caution should be used in reporting a diagnostic culture negative when only solid forms are found. Such cultures should be incubated a longer period of time and subcultures studied before a negative report is made. If necessary, tests for virulence would seem to be advisable. To report such a culture negative, a priori, merely because the morphology fails to conform to that of the accepted virulent types, is, in view of the observations reported here and elsewhere, hazardous.

2. THE RELATION OF MORPHOLOGY TO VIRULENCE

It is common practice in diagnostic laboratories to regard the solid staining bacilli of the diphtheria group and certain of the barred and granular types as being avirulent and to render a negative report on cultures which show only these forms. In view of numerous instances in which strains have been reported to change their morphology from time to time under certain conditions, a study of these strains seemed advisable.

Bunker [4] reports an observation of a single strain which showed all the morphologic types at different intervals. Meader,[5] in a study of 25 strains, found variations in morphology and virulence in subcultures, and concluded that the virulence of a strain is not closely correlated with the morphology. In the series of strains just described by me, variations were found to occur among certain groups. Wayson [6] found that solid staining bacilli were sometimes virulent, and states that morphology alone is an insufficient index of virulence.

This study was undertaken to determine the virulence of certain solid staining strains, both those that remained solid at all observations and those that on longer incubation and prolonged growth became granular or bipolar in their morphology. Since the change from uniformly staining organisms to those with granules depends, in some cases at least, on the age and the products of growth of the culture, it was interesting to determine whether those cultures which eventually become typically granular were virulent.

It was shown in the preceding section that since there is a tendency for solid staining forms to develop on further cultivation into the characteristic type C organisms, care should be taken in studying such cultures and the virulence of the individual cultures should, if necessary, be determined before they are reported as negative.

Table 3 shows the results of virulence tests with guinea-pigs with strains which were initially of types D_2 and E_2. With the first six strains of the table the tests were made by the subcutaneous method using 1 c c of culture. With the last five strains the intracutaneous method was used.

It is evident that many of the uniformly staining strains are virulent for guinea-pigs. It is interesting to note that strains 222 and 223, which were originally solid but which on longer cultivation developed granular characteristics, remained avirulent.

[4] Abstracts of Bacteriology, 1, p. 31, 1916.
[5] Jour. Infect. Dis., 1919, 24, p. 145.
[6] Public Health Reports, 1916, 31, p. 3113.

TABLE 3

VIRULENCE TESTS WITH STRAINS INITIALLY OF TYPES D_2 AND E_2

Culture	Morphology		Result		
	Diagnostic Culture	Pure Culture	24 Hours	48 Hours	72 Hours
181	D_2	D_2	.....	.....	Death
219	D_2	D_2	Local edema	.....	Death
221	D_2	C	Negative	Local edema	Death
222	D_2	C	.....	Negative	
223	D_2	C	.....	Negative	
224	D_2	C	.....	.....	Marked edema of abdominal wall
226	D_2	D_2	+	++++	++++
227	D_2	C	++	++++	++++
228	D_2	C	++	++++	++++
229	D_2	D_2	Negative	++	++
230	D_2	D_2	Negative	Negative	Negative

From the evidence presented here and elsewhere, it seems that morphology bears little relation to toxin production. It is undoubtedly true that the greater proportion of virulent strains show granular or bipolar staining characteristics and are the typical forms of diphtheria bacilli in that they occur in by far the greater number of instances. However, to regard so-called solid staining forms as avirulent on the basis of morphology and staining characteristics as the sole criteria, is open to serious question.

Weaver [7] found, from a study of a series of strains isolated from patients and from contacts, that the majority of these strains are virulent. No observations are recorded concerning the morphology of these strains, but his conclusion is that such strains are usually virulent. A much better criterion of virulence, from the standpoint of prevention, is the history of the carrier state. If a history of contact with an active case is obtained, the strain should be regarded as virulent until proved otherwise. If there are cases in which no history of such contact can be obtained—usually a small minority of cases—a test for virulence should be made regardless of morphology before such individuals are allowed to become a potential menace to the community. It would seem that morphology as a criterion of virulence has no more foundation in connection with the diphtheria bacillus than does the morphology of the pneumococcus, for example, in ascertaining its virulence.

To determine the virulence of the strain harbored by a carrier requires the detention of the individual for from 48-72 hours longer, but this inconvenience is more than compensated by the benefit to the

[7] Jour. Infect. Dis., 1917, 20, p. 145.

community from a definite knowledge of the virulence or lack of virulence of the organisms which he carries. In the case of a carrier with a definite history of contact with a patient the organisms should be considered virulent until proved otherwise. It is far better to cause one individual a slight inconvenience than to run the risk of inflicting unnecessary cases on the community. In reporting on release cultures after convalescence from diphtheria, the morphology should bear still less weight. A negative report based on the fact that the culture shows only solid forms at the time of examination should not be made until the virulence has been determined. The fact that the source is a recently active case should carry more influence than the staining properties.

3. BIOLOGIC GROUPS AS EVIDENCED BY AGGLUTINATION TESTS

Attention has been primarily devoted, in immunologic studies of B. diphtheriae, to the toxin and the production of antitoxin. The unity of all strains in their immunologic properties has been assumed rather than definitely determined and, in consequence, a single strain, if it forms a potent toxin, is considered sufficient for the production of antitoxin for therapeutic purposes.

The few studies of the agglutinability of the diphtheria bacillus which have been made in the past have shown certain variations in this respect between different strains of the organism. Langer,[8] using a monovalent serum, found that certain strains were not agglutinated. These nonagglutinating strains were otherwise typical diphtheria bacilli and he states that they represent, clinically, cases of mild infection and healthy carriage. Mason [9] used the agglutination test as a means of diagnosis and found in a study of 65 strains that, with a serum of relatively low titer (1:320), differences in degree of agglutinability exist among the individual strains.

It appears, then, that differences in immunologic properties exist among the various strains of the diphtheria bacillus. In order to determine the degree and specificity of these differences, a series of 206 different strains has been studied with regard to their agglutinability. These represent diagnostic cultures from acute cases of diphtheria, release cultures and cultures from healthy carriers. The morphologic types represented in the series were quite universal, most of the types given in Wesbrook's classification being included. As

[8] Centralbl. f. Bakteriol., II, 78, p. 117, 1916.
[9] Military Surgeon, 1919, 45, p. 560.

pointed out in the preceding part on the specificity of the morphologic groups as evidenced by their tinctorial properties, it was found that the morphology tends to vary, the tendency, in brief, being toward the granular types, especially in old cultures. Consequently, in the study of the agglutinability of the strains in this series detailed attention was not paid to the morphologic characteristics. It was found, however, that morphology bears no observed relation to agglutinating properties.

A monovalent serum was produced in rabbits, using for its production, a granular form corresponding to type C. This strain had shown no variations in morphology, being typically granular at all observations. The growth of a blood serum slant, incubated for 24 hours and suspended in 10 c c of salt solution, was used for injecting the rabbit, the injections being made intravenously in increasing amounts. An agglutinating serum was obtained in this way with a titer of 4,860 for the homologous strain. Using this serum, the members of the series were tested for their agglutinating properties.

Of the series of 206 strains, 169 were found to agglutinate with this monovalent serum; 37 failed to agglutinate. The agglutinating members of the series showed no variations in degree of agglutinability. All were agglutinated in a dilution of serum of 1:4,860 as did the strain used in the production of the serum. The 37 strains which failed to agglutinate in any dilution of serum showed no differences morphologically or culturally from the agglutinating strains.

A member of this group of nonagglutinating strains was used to produce a second agglutinating serum in a rabbit. This serum also had a titer of 4,860 or higher for the homologous strain. Using this serum, agglutination tests were made on the members of the series which failed to agglutinate with the first serum. All of these strains were found to agglutinate with this serum in high dilution (1:4,860).

Judging by the evidence furnished by the agglutination test there are two biologic groups of the diphtheria bacillus, one including 82% of the series of 206 strains studied, the other containing the remaining 18%. No evidence of cross-agglutination was found among the members of this series. The specific agglutinating serum for the second group showed no agglutinating power for any of the members of the first group. Members of the second group were likewise not agglutinated in any dilution by the first group serum. In this respect the two groups are distinct.

The members of the second group show no peculiarities of morphology, staining properties or toxin production, which differentiate them from strains of the first group. Typical granular organisms of high virulence for guinea-pigs are found in both groups and the proportions of the morphologic types are about the same. No marked preponderance of any one form is found in either group.

As pointed out already, solid forms occur which possess a high degree of virulence for guinea-pigs. Such strains have been found to exist in both of these groups. These strains in the majority of instances eventually show granules and swollen forms characteristic of the diphtheria bacillus. Some, however, retain their solid staining characteristics indefinitely. There seems to be no more reason for using tinctorial characteristics as a criterion for virulence than for using them as a guide to the serologic grouping.

There seems, furthermore, to be no reason for believing that the members of the second group should show any marked differences in virulence as compared with the first. As a matter of fact, of 24 strains of the second group which were tested, 18 were found to be highly virulent for guinea-pigs. It is necessary to show, in order to prove that a case of an infectious disease is the result of contact with a particular carrier, that the two strains are identical serologically. Consequently, it is highly improbable that a serologic group should exist composed entirely of avirulent organisms. Serologic properties are independent of virulence, rather than dependent on that property.

In this series of 206 strains no individuals were encountered which were inagglutinable. All the members of the series fell into one of the two groups with no differences observed in degree of agglutinability. As judged by the results of agglutination, two groups exist which include all strains of the diphtheria bacillus.

4. THE PROTECTIVE PROPERTIES OF DIPHTHERIA ANTITOXIN WITH REFERENCE TO BIOLOGIC GROUPS

It seems from the preceding evidence that two definite groups of the diphtheria bacillus exist, in so far as agglutinins are concerned. The experiments described in this section deal with the relationships of other properties of the two groups, notably their toxins and the corresponding antitoxins. It does not necessarily follow that other antigenic properties would be analogous to those responsible for agglutinin formation. The protective properties of standard diphtheria antitoxin are well known, and it was consequently deemed advisable to test the protective properties of this antitoxin against strains of the two groups which were ascertained by the agglutination test.

Besides the standard antitoxin obtained from the Hygienic Laboratory of the U. S. Public Health Service, through the courtesy of the director, Dr. G. W. McCoy, specimens of antitoxin were obtained

from six different commercial houses. All of these were tested against a culture of the strain of diphtheria bacillus known as "Park No. 8," which is the strain used extensively in the production of antitoxin and which was found by agglutination to fall into the first or larger group. The protective properties of these different brands of antitoxin against this strain were found to be effective and the number of units per c c essentially as stated on the label.

Experiments were then made to determine the comparative value of the protective properties of these various antitoxins against strains of the second group as well. In the following protocols, unless otherwise stated, the amount of culture injected is that amount of a 24-hour blood serum slant suspended in 5 c.c. of salt solution.

G. pig 1. Control. 1.0 c c subcutaneously of culture 210 (group 1). Dead in 48 hours. Lesions characteristic of diphtheria.

G. pig 2. 1.0 c c subcutaneously of culture 210 (group 1). 100 units antitoxin 18 hours previously. Remained normal.

G. pig 3. Control. 1.0 c c subcutaneously of culture 221 (group 2). Dead in 36 hours. Lesions characteristic of diphtheria.

G. pig 4. 1.0 c c of culture 221 (group 2). 100 units of antitoxin 18 hours previously. Dead in 36 hours. Lesions characteristic of diphtheria.

G. pig 5. 1.0 c c of culture 141 (group 2). Dead in 48 hours. Lesions characteristic of diphtheria.

G. pig 6. 1.0 c c of culture 141 (group 2). 100 units of antitoxin 18 hours previously. Dead in 48 hours. Lesions characteristic of diphtheria.

From this experiment, using two strains of group 2 organisms, there seems to be no evidence that any degree of protection was furnished against strains of group 2 by the standard antitoxin which protected satisfactorily against a virulent strain of group 1. It is at least probable from this experiment that two of the group 2 cultures differ from the group 1 organisms in their toxin production as evidenced by protective tests.

It seemed desirable to test the protective property of the standard antitoxin against a representative number of the members of group 2 and for this experiment, in order to conserve guinea-pigs, the intracutaneous method [10] was used. Previous experience has shown this method to be satisfactory for virulence tests, and it has the advantage that several tests can be made simultaneously on the same animal. The following table shows the results with 18 strains of group 2 and 6 of group 1 to serve as controls.

[10] Zingher, A., and Soletsky, D.: Jour. Infect. Dis., 1915, 17, p. 455.

TABLE 4

RESULTS OF TEST

No. of Culture	Group by Agglutination	Result in 72 Hours—Degree of Local Edema and Necrosis	
		Antitoxin	No Antitoxin
173	1	0	++
59	1	0	++++
223	1	0	++++
34	1	0	++++
119	1	0	++
24	1	0	++++
185	2	++++	++++
207	2	++++	++++
118	2	++++	++++
170	2	++++	++++
83	2	++++	++++
74	2	++++	++++
69	2	++	++++
60	2	++++	++++
57	2	++	++
53	2	++++	++++
43	2	++	++++
40	2	++++	++++
208	2	+	++
181	2	+	+
219	2	++++	++++
168	2	++++	++++
141	2	++++	++++
95	2	++++	++++

The results obtained by subcutaneous injection are thus corroborated by intracutaneous tests with a larger series of strains. It is seen from the table, however, in the case of two strains, 69 and 208, that while a certain amount of necrosis was produced at the site of inoculation, it was not so marked as in the control animal. However, in these experiments a large amount, 100 units, of antitoxin was used, a much larger amount than is necessary to protect a guinea-pig against the amount of culture injected. Consequently in order to determine more accurately the relative differences between the two groups and to graduate the dosage more nicely, in the following experiments the pure toxin was used instead of the culture of the bacilli. Two strains were selected from each group, strains which were found to produce a potent toxin, the MLD for the toxin produced by these cultures being found to be from 0.005-0.01 c c. With these toxins the following experiment was made.

G. pig 1. Injected subcutaneously with the following mixture: 2 MLD toxin 34 (group 1) and 10 units standard antitoxin, incubated 2 hours at 37 C. Remained normal.

G. pig 2. Control. Injected subcutaneously with the following mixture: 2 MLD toxin 34 (group 1) and 1.0 c c salt solution incubated 2 hours. Guinea pig dead in 48 hours.

G. pig 3. Injected subcutaneously with the following mixture: 2 MLD toxin 74 (group 2) and 10 units standard antitoxin, incubated 2 hours. Dead in 48 hours.

G. pig 4. Control. Injected subcutaneously with the following mixture: 2 MLD toxin 74 (group 2) and 1.0 c c salt solution. Dead in 48 hours.

G. pig 5. Injected subcutaneously with the following mixture: 2 MLD toxin 53 (group 2) and 10 units standard antitoxin. Dead in 72 hours.

G. pig 6. Control. Injected subcutaneously with the following mixture: 2 MLD toxin 53 (group 2) and 1.0 c c salt solution. Dead in 72 hours.

It appears from these results that guinea-pigs injected with the toxin of strains of group 2 are not protected by five times the corresponding amount of standard antitoxin. It was thought possible that larger amounts of antitoxin might show a protective action, especially if group antitoxins were found to be present in highly potent antitoxins. In order to determine whether this were so a series of guinea-pigs were injected with increasing amounts of antitoxin, the amount of toxin remaining constant — two minimal lethal doses being the amount injected. The following protocol illustrates the results obtained.

G. pig 1. Control. 2 MLD toxin 34 (group 1) and 5 units standard antitoxin. Survived.

G. pig 2. 2 MLD toxin 74 (group 2) and 5 units standard antitoxin. Dead in 48 hours.

G. pig 4. 2 MLD toxin 74 and 20 units antitoxin. Marked local edema and toxemic symptoms. Survived.

G. pig 5. 2 MLD toxin 74 and 25 units antitoxin. Local edema. Survived.

G. pig 6. 2 MLD toxin 74 and 50 units antitoxin. Survived. No local reaction.

There seems, therefore, to be present in the standard antitoxin a certain amount of group antitoxin which protects against both groups of the diphtheria bacillus. This is not present apparently in sufficient amounts to exert its action until many times the number of units of antitoxin as of toxin are present. Furthermore, different brands of antitoxin vary in their content or their titer in respect to this characteristic. Two antitoxins, designated "A" and "D," failed to show this protective action until 100 units were injected for every MLD. One antitoxin, (C), failed to protect up to 1,000 units, which was as far as the experiment was carried.

Evidence having been obtained that the standard diphtheria antitoxin showed group antitoxins in small amounts the question arose whether an antitoxin produced with a group 2 strain of the diphtheria bacillus would likewise show this characteristic, possibly more mark-

edly. In other words, group antitoxins might be present in higher titer. A rabbit was injected with gradually increasing amounts of group 2 toxin and also virulent cultures of group 2 bacilli. However, a highly potent antitoxin was not obtained. The serum from this animal had a titer of about 50 units per c c. While this was a relatively low titer no evidence was obtained that group agglutinins were developed in any greater concentration than in the standard antitoxin. The following experiment illustrates the relative protective properties of the two group antitoxins:

G. pig 1. 1 MLD toxin 34 (group 1) and 5 units standard antitoxin. Survived. No local reaction.

G. pig 2. 1 MLD toxin 53 (group 2) and 5 units standard antitoxin. Dead in 96 hours.

G. pig 3. 1 MLD toxin 34 (group 1) and 5 units group 2 antitoxin. Dead in 96 hours.

G. pig 4. 1 MLD toxin 53 (group 2) and 5 units group 2 antitoxin. Survived. Slight local edema.

G. pig 5. Control. 1 MLD toxin 34 (group 1). Dead in 96 hours.

G. pig 6. Control. 1 MLD toxin 53 (group 2). Dead in 96 hours.

It seems from the experimental evidence that the toxins produced by the two groups of diphtheria bacillus, in so far as can be judged by the corresponding antitoxins, are not so sharply defined as are the agglutinins. No evidence was obtained of agglutinins common for both groups. Strains which were agglutinated by one agglutinating serum were not agglutinated by the serum of the other group even in low dilution (1:20). The standard antitoxic serums, however, show some evidence of containing antitoxins common for both groups. These are not, it is true, present in any large quantities, but undoubtedly they occur to some extent. The minimum amount of the standard antitoxin which was found to protect against one minimal lethal dose of the group 2 toxin was 20 units and 5-10 units regularly failed to protect.

The results of these experiments may throw some light on those cases of diphtheria which are not benefited except by large amounts of antitoxin. It is entirely possible that such cases are due to infection with a strain belonging to the second or smaller group of the diphtheria bacillus and consequently a large amount of the standard antitoxin is necessary to obtain the benefit of the small amount of antitoxin common to both groups.

Furthermore, the death rate from diphtheria, in spite of the increasing use of antitoxin and in spite of its use early in the disease, is still about 10%.[1] How many of such fatal cases are due to infection with organisms of group 2 it is, of course, at the present time, impossible to say. A study of these stubborn cases which fail to yield to liberal doses of antitoxin would seem advisable and might throw light on the use of antitoxin.

In view of the experimental evidence here set forth it would seem that the inclusion of a representative of group 2 in the production of therapeutic antitoxin would greatly increase its efficiency and should be an influence in lowering the mortality of diphtheria in proportion to the frequency of the occurrence of strains of group 2. The occurrence of such strains is, judging from the series of 206 strains studied, about 18%.

It is possible that the use in the Schick test of a mixture of the two toxins including both groups will make this test of more universal value. An individual may be immune to infection with the larger group and yet be susceptible to members of group 2. It is probable, however, that the antitoxin which such an individual possesses contains certain group protection properties similar to the artificially produced antitoxin in the horse. On the other hand, if the antitoxin is not present in considerable amounts the experimental evidence suggests that the protection against the second smaller group will be inadequate. That immunity to infection with group 1 protects against all infection with diphtheria for practical purposes is probable, due to the fact that the second group occurs more rarely.

Likewise, active immunization with toxin-antitoxin mixtures would probably be made more complete by the use of mixtures of the two group toxins and antitoxins or a polyvalent antitoxin. That some protection is furnished against group 2 by the production of a high degree of immunity against group 1 is probably true. The degree of protection, however, against the second group is problematic and at best, is incomplete.

The presence of group antitoxins in certain small amounts in the standard antitoxin saves it from being valueless in group 2 infections, but in all cases, its value for such infections could be enhanced by the inclusion in its production of group 2 toxin.

[1] Carey, B. W.: Bost. Med. and Surg. Jour., 1919, 181, p. 92; Public Health Reports, 1919, 34, p. 1063.

SUMMARY

The morphologic characteristics of the diphtheria bacillus show a tendency to variations from time to time. The morphologic types are, therefore, apparently nonspecific. The solid forms corresponding to types D_2 and E_2 are probably young forms of the more common granular types.

Solid staining types of the diphtheria bacillus are sometimes virulent. They should not be regarded as avirulent on the basis of morphology alone. Virulence tests should be made to determine the status of carriers.

Instead of placing undue emphasis on morphology, more attention should be paid to the history of the carrier state, whether a convalescent or a contact with an active case. Such cultures should be considered virulent, regardless of morphologic characteristics, until proved otherwise.

By the use of the agglutination test two groups of the diphtheria bacillus have been determined. These groups are distinct, showing no evidence of cross-agglutination.

The members of the two groups show no differences in morphology or in relative virulence.

The results of agglutination tests in determining two groups of the diphtheria bacillus are corroborated by protective tests with antitoxin against the two group toxins.

Evidence is presented showing that the antitoxins of these groups are not so sharply differentiated as are the agglutinins. Group antitoxins seem to exist in small amounts common to both groups.

The effectiveness of therapeutic diphtheria antitoxin could probably be enhanced by the inclusion in its production of a member of the second or smaller group.